Center for Toxicology and Environmental Health, L.L.C.

4301 W. Markham St. Suite 100A Hendrix Hall Mail Slot 767 Little Rock, AR 72205
Ph. (501)614-2834 Fax (501)614-2835 Website www.cteh.uams.edu

November 30, 1998

NTP Board of Scientific Counselors
Report on Carcinogens Subcommittee
U.S. Department of Health and Human Services
Public Health Services
National Toxicology Program
Research Triangle Park, NC 27709

Regarding: Comments to the Draft Report on Carcinogens-Background Document for Environmental Tobacco Smoke

Dear Sirs:

Thank you for the opportunity to provide these comments regarding the Draft Report on Carcinogens-Background Document for Environmental Tobacco Smoke prepared by Technology Planning and Management Corporation for the U.S. Department of Health and Human Services.

My comments that are attached to this letter address the use of the A/J mouse as a screening tool in chemical carcinogenesis. The draft report gives considerable attention to studies of sidestream smoke in A/J mice. Nearly all of the section entitled "4. Studies of Cancer in Experimental Animals" is a discussion of A/J mouse studies. I have used the term "Strain A mouse" to include the A/J substrain as well as all other substrains of the Strain A mouse.

I am concerned that the draft document fails to place the Strain A mouse studies in proper perspective for an overall determination of chemical carcinogenesis, including the relevance of Strain A mouse studies to humans. The Strain A mouse is highly susceptible to spontaneously occurring lung tumors. At 18 months of age, the incidence of spontaneous lung tumors is approximately 90%, and there is a nearly 100% lifetime incidence.

Also, there is no human neoplastic equivalent to the benign mouse lung adenoma, which is the principal tumor type that develops in the Strain A mouse bioassay system. Both of these features limit the value of the model for predicting human response or human risks to potential chemical carcinogens.

[redacted]

In my enclosed comments, I have provided some regulatory perspective regarding the relative worth of the Strain A mouse model to an overall determination of carcinogenicity. From the US regulatory perspective, the Strain A mouse bioassay is viewed as a screening tool and should not be considered a substitute for chronic rodent bioassays.

My comments also discuss specific examples of the United States regulatory perspective on the relative value of the Strain A mouse studies for "weight of evidence" classifications of chemical carcinogens. Based on many "weight of evidence" chemical carcinogenicity determinations by the United States Environmental Protection Agency (USEPA) where Strain A mouse results were available, Strain A mouse studies carry little weight in the overall determination of a chemical's carcinogenicity to humans. In a case where only positive Strain A mouse data were available, the USEPA determined that the Strain A mouse data were "inadequate" to conclude that the chemical was a potential carcinogen in humans (e.g., 2-chlorobutane).

Lastly, my comments address specific Strain A mouse studies cited in the draft report. Tobacco smoke treatment results in Strain A mouse lung tumors only at concentrations that also result in cell proliferation. It is therefore possible that the lung tumors may be caused by cell proliferation that occurs only at very high, environmentally irrelevant levels of exposure. Lower, more relevant exposures do not result in cell proliferation and therefore, are unlikely to result in an increased incidence of lung tumors.

Once again, I appreciate the opportunity to provide the NTP Board of Scientific Counselors with these comments.

Sincerely

Center for Toxicology and Environmental Health

Glenn C. Millner/jo

Glenn C. Millner, Ph.D.
Senior Toxicologist

[redacted]

Comments Regarding the Draft Report on Carcinogens Background Document for Environmental Tobacco Smoke
Prepared by Glenn C. Millner, Ph.D.

United States Regulatory Perspective on the Use of Strain A Mouse Studies

Over the last 13 years, regulatory guidance consistently has advocated the cautious use of results from the Strain A mouse bioassay. The primary use of data from these studies has been to support the results of more thorough chronic bioassays. Although hundreds of chemicals have been evaluated in the Strain A mouse bioassay, none of these data have been used quantitatively in human risk assessment. Thus, the Strain A mouse bioassay has a limited supportive role in providing additional qualitative data regarding a potential chemical carcinogen.

Regulatory guidance regarding the use of Strain A mouse adenoma bioassay results has been available since at least 1985 when the United States Office of Science and Technology Policy (OSTP) published its framework for assessment of cancer risks posed by chemicals. As discussed in its summary of the document, the 1985 OSTP report "is the culmination of drafts extensively reviewed by governmental and non-governmental scientists." It represented the mainstream of scientific thought regarding chemical carcinogenesis and human risk assessment of chemical carcinogens.

The OSTP regarded the Strain A mouse bioassay as a screening tool for identifying potential chemical carcinogens:

> Several short-term in vivo bioassays to detect chemical carcinogens and/or promoters have been developed. Of these assays, the mouse skin papilloma system and the Strain A mouse lung adenoma have been used for testing while the liver foci bioassay is still viewed as intermediate in biological relevance between *in vitro* systems and the long-term animal bioassay because *in vivo* metabolism and/or chemical disposition are provided for by *in vivo* exposure. However, as with the *in vitro* tests, the relevance of the endpoints measured to the development of malignant tumors remains an open question. (page 41)

Specifically in regard to the Strain A mouse bioassay, the OSTP stated:

> The system has been criticized because the adenoma is a benign growth and an analogous tumor does not appear in humans. (page 41)

Thirteen years later, the OSTP questions regarding the relevance of the Strain A mouse bioassay to humans remain unanswered.

In 1996, the USEPA published an update to its 1986 Guidelines for Carcinogen Risk Assessment. This guidance, entitled the "Proposed Guidelines for Carcinogen Risk Assessment" also addresses use of data from Strain A mouse bioassays. It is worthwhile

to note that over that time, regulatory guidance regarding the use of the Strain A mouse bioassay has not changed.

The USEPA Proposed Guidelines for Carcinogen Risk Assessment more recently recognized the limitations of studies like the Strain A mouse bioassay, stating:

> One needs to recognize the limitations of these experimental protocols such as short duration, limited histology, lack of complete development of tumors, or experimental manipulation of the carcinogenic process that may limit their contribution to the overall assessment. Generally, their results are appropriate as aids in the assessment for interpreting other toxicological evidence (e.g., rodent chronic bioassays), especially regarding potential modes of action. (page 55, USEPA, 1996)

Thus, the role of the Strain A mouse bioassay in cancer risk assessment has changed little over the last 15 years; past and current regulatory guidance emphasize the screening nature of the test and its minor overall contribution to "weight of evidence" evaluations of chemical carcinogenicity.

Recent Regulatory Use of Strain A Mouse Studies in Determining Chemical Carcinogenicity

The results of my recent review of the use of the Strain A mouse bioassay results is in keeping with OSTP guidance and USEPA's Proposed Guidelines for Carcinogen Risk Assessment. The USEPA's Integrated Risk Information Service (IRIS) database summarizes toxicity and carcinogenicity information for hundreds of chemicals. "Weight of evidence" reviews of the carcinogenicity of many chemicals are contained in the IRIS database. The IRIS database is updated as new information becomes available for review. Thus, the IRIS database reflects current regulatory policy concerning the possible carcinogenicity of chemicals to humans.

As the starting point for my evaluation, I used Stoner's list of 117 chemicals tested in the Strain A mouse bioassay and his determination of whether these studies were positive or negative with regard to tumorigenicity (Stoner, 1991). I compared Stoner's list to the IRIS database to determine whether USEPA had completed an evaluation of the carcinogenicity of those chemicals. I found that USEPA had evaluated 20 of the chemicals tested by Stoner on its IRIS database. These chemicals are listed in Table 1.

Of the 20 chemicals listed in Table 1, 15 are considered to be "known" (Group A), "probable" (Group B2), or "possible" (Group C) human carcinogens. Of these 15 chemicals, the USEPA has derived carcinogenic potency estimates (termed slope factors) for 13 of these chemicals. In no case were the Strain A mouse bioassay results used in determining a numerical estimate of cancer potency, indicating that as per the OSTP guidance and Proposed Guidelines for Carcinogen Risk Assessment, the Strain A mouse

bioassay results are used in a supportive rather than primary role in evaluating chemical carcinogenicity. Four of the chemicals listed by the USEPA as probable or possible human carcinogens (chloroform, epichlorohydrin, 1,1,2,2-tetrachloroethane, and 1,4-dioxane) were negative in the Strain A mouse bioassay.

When positive carcinogenicity data are available only from the Strain A mouse bioassay, the USEPA has determined that these data are inadequate to make a determination of the carcinogenicity of the chemical. One example of this is the USEPA interpretation of the Strain A mouse bioassay results for sec-butyl chloride (2-chlorobutane). Although this chemical was positive in the Strain A mouse bioassay, USEPA considered this evidence to be inadequate to make a determination of possible or probable human carcinogenicity:

> In a study designed to look only for lung tumors after short-term, high exposure, Poirier et al. (1975) gave groups of 10 male and 10 female Strain A/Heston mice a total of 13 intraperitoneal injections (3 injections/ week) of 7, 17.5, or 35 mmol/kg (648, 1620, or 3240 mg/kg) 2-chlorobutane in tricaprylin. Untreated and tricaprylin-treated mice were used as negative controls, and urethane-treated mice were used as positive controls. Survival of animals in the low-, mid-, and high-dose groups was 75, 75, and 50%, respectively. In the untreated and vehicle controls and urethane-treated group, survival was >90%. Remaining animals were sacrificed 24 weeks after the first injection. 2-Chlorobutane induced a dose-related increase in the average number of lung tumors per mouse that was statistically significantly elevated at the 35 mmol/kg (3240 mg/kg) dose level and in the urethane-treated positive control group relative to untreated and vehicle controls. This type of study is generally regarded as a short-term in vivo screening bioassay. (Section II.A.3. Animal Carcinogenicity Data section of IRIS database for 2-Chlorobutane accessed November 23, 1998)

Despite the observation of a statistically significant increase in lung tumors per mouse and a dose-related increase in lung tumors per mouse, the USEPA declared the Strain A mouse bioassay data "inadequate" for a positive determination of the carcinogenic potential of sec- butyl chloride. This position is consistent with OSTP's 1985 guidance and USEPA's recent guidance regarding the interpretation of Strain A mouse bioassay results.

In regulatory practice, Strain A mouse bioassay results are viewed as providing supportive but not primary evidence of chemical carcinogenicity. When faced with only positive Strain A mouse bioassay results, regulatory agencies such as the USEPA are unlikely to classify a chemical as a potential human carcinogen without more conclusive results that are available from chronic rodent bioassays.

[redacted]

Specific Comments Regarding Interpretation of the Strain A Mouse Results

Exposure of the strain A mouse to tobacco smoke has resulted in inconsistent and equivocal findings. Initial testing in the 1940's (Lorenz et al., 1942) using extremely the high particle concentration of 1,000 mg/m^3 failed to increase lung tumors. Subsequent efforts by Essenberg and colleagues in the 1950's (Essenberg, 1952; Essenberg et al., 1955; Essenberg, 1956) produced mixed evidence for increased tumors in the Strain A mouse lung. Unfortunately, adequate exposure information was lacking from these works. A more recent 1996 report by Finch et al., exposing Strain A mice to 248 mg/m^3 particle concentrations for 26 weeks detected no increased lung tumors in exposed mice. In addition, mice pretreated with the initiator, NNK, failed to develop enhanced tumor incidence or multiplicity.

Witschi and colleagues have reported the results of a series of studies using the Strain A mouse exposed to aged and diluted sidestream smoke (Witschi et al., 1998). In the initial experiments mice were exposed to 4.1 mg/m^3 of particulate for 6 months, and there were no differences in lung tumor incidence or tumor multiplicity (Witschi et al., 1995). Cell proliferation was significantly altered only in nasal passages, and only during the early phases of exposure. Subsequent experiments using 87 mg/m^3 exposure for 5 months detected an increased incidence and multiplicity of Strain A mouse lung tumors (Witschi et al., 1997). However, no dose-response was observed. Animals exposed for 5 months to 87 mg/m^3 or for 2.5 months to 53 mg/m^3 developed similar numbers of tumors.

In the Witschi study, cell proliferation was significantly increased in the alveolar zone during the first 2 weeks of exposure, and a similar transitory increase in labeling indices was observed in the large airways and terminal bronchioles. These studies do not adequately separate the issue of cell proliferation versus direct carcinogenicity to the lung epithelial cells. It is possible that a chemically induced increased rate of proliferation leads to the more rapid rate of appearance of the relatively high incidence of naturally occurring lung tumors seen in older Strain A mice.

As Counts and Goodman have pointed out (1995), dose selection in a bioassay influences the mechanism of carcinogenic response, and over a wide range of doses the mechanism changes with changing dose. Thus, a carcinogenic effect observed at a high dose that results in an increase in cell proliferation may not be indicative of a human response at lower, more environmentally relevant doses. Increased cell proliferation may facilitate carcinogenesis due to the fact that mitogenesis can facilitate mutagenesis. When a cell divides, an unrepaired DNA lesion has a certain probability of yielding a mutation. The degree to which the dose of a chemical increases the rate of cell division above background in an important determinant of its capacity to act as a mutagen and facilitate carcinogenesis.

The Strain A mouse lung tumors appear particularly susceptible to this phenomenon since the high rate of spontaneous tumors is linked to three fragile pulmonary adenoma

susceptibility genes (*Pas*). These genes include the proto-oncogene, K-*ras*, and the H-2 histocompatibility locus and genes which regulate the basal proliferative rate of cell from which the adenomas arise. Recent work by Matzinger et al. (1997) demonstrated a tissue-specific higher level of K-*ras* transcripts in lung tissue of Strain A mice, which may lead to preferential selection of cells with a mutant allele instead of the tumor resistant allele, and could lead to a more rapid clonal expansion of these cells. Thus, a high rate of spontaneous mutations to these loci contributes to the high spontaneous tumor rate. Any increase in cell proliferation would be expected to enhance the number of fixed mutational lesions to these sites. Thus, increased mitogenesis may facilitate mutagenesis and lead to an increase tumor incidence in the Strain A mouse model. Such a mechanism would suggest a threshold exposure level below which no mitogenesis and therefore, no increased incidence of tumors would occur. Consequently, the relevance of the Strain A mouse bioassay and therefore, the results of the Witschi studies to effects in humans must be questioned.

References

Counts, J.L. and Goodman, J.I. 1995. Principles underlying dose selection for, and extrapolation from, the carcinogen bioassay: dose influences mechanism. Reg. Toxicol. Pharmacol. 21: 418-421.

Essenberg, J.M. 1952. Cigarette smoke and the incidence of neoplasm of the lung in the albino mouse. Science 116:561-562.

Essenberg, J.M., Horowitz, M., and Gaffney, E. 1955. The incidence of lung tumors in albino mice exposed to the smoke from cigarettes low in nicotine content. West. J. Surg. Obstet. Gynecol. 63:265-267.

Essenberg, J.M. 1956. The effects of arsenic in tobacco on primary neoplasms of the lungs of albino mice. West. J. Surg. Obstet. Gynecol. 64:35-36.

Lorenz, E., Stewart, H.L., Daniel, J.H., and Nelson, C.V. 1942. The effects of breathing tobacco on strain A mice. Cancer Res. 3:123.

Matzinger, S. A.; Chen, B.; Wang, Y.; Crist, K. A.; Stoner, G. D.; Kelloff, G. J.; Lubet, R. A., and You, M. Tissue-specific expression of the K-ras allele from the A/J parent in (A/J x TSG-p53) F1 mice. Gene. 1997 Apr 1; 188(2):261-9.

OSTP (Office of Science and Technology Policy). 1985. Chemical Carcinogens; A Review of the Science and Its Associated Principles, February 1995. March 14, 1995 Federal Register, pages 10371-10442.

Poirer, L.A., Stoner, G.D., and Shimkin, M.B. 1975. Bioassay of alkyl halides and nucleotide base analogs by pulmonary tumor response in strain A mice. Cancer Res. 35:1411-1415.

Rajini, P. and Witschi, H. Short-term effects of sidestream smoke on respiratory epithelium in mice: cell kinetics. Fundam Appl Toxicol. 1994 Apr; 22(3):405-10.

Stoner, G. D. Lung tumors in strain A mice as a bioassay for carcinogenicity of environmental chemicals. Exp Lung Res. 1991 Mar-1991 Apr 30; 17(2):405-23.

USEPA (United States Environmental Protection Agency) 1996. Proposed Guidelines for Carcinogen Risk Assessment. Office of Research and Development. April 1996.

Witschi, H., Oreffo, V.I.C., and Pinkerton, K.E. 1995. Six-month exposure of strain A/J mice to cigarette sidestream smoke: cell kinetics and lung tumor data. Fundam. Appl. Toxicol. 26:32-40.

Witschi, H.; Espiritu, I.; Peake, J. L.; Wu, K.; Maronpot, R. R., and Pinkerton, K. E. The carcinogenicity of environmental tobacco smoke. Carcinogenesis. 1997 Mar; 18(3):575-86.

Witschi, H. Tobacco smoke as a mouse lung carcinogen. Exp Lung Res. 1998 Jul-1998 Aug 31; 24(4):385-94.

[redacted]

Table 1
Comparison of Strain A Mouse Bioassay Results from Stoner (1991)
to USEPA's Determination of Carcinogenicity

Chemical	[1]Test result (Stoner, 1991)	[2]USEPA Group	[3]USEPA Numerical Estimate of Carcinogenic Potency in Humans?	Strain A mouse bioassay data used for quantitative extrapolation of carcinogenic potency to humans?
Benzo[a]pyrene	+	B2	Yes	No
Dibenz[a,h]anthracene	+	B2	No	No
Benz[a]anthracene	+	B2	No	No
Ethyl carbamate	+	--	No	No
N-Nitrosodimethylamine	+	B2	Yes	No
N-Nitrosodiethylamine	+	B2	Yes	No
N-Nitrosodibutylamine	+	B2	Yes	No
Methyl iodide	+/-	--	No	No
Bromoform	+	B2	Yes	No
Chloroform	-	B2	Yes	No
1,2-Dichloroethane	+	B2	Yes	No
Dichloromethane	+	B2	Yes	No
Epichlorohydrin	-	B2	Yes	No
1,1,2,2-Tetrachloroethane	-	C	Yes	No
sec-Butyl chloride (2-Chlorobutane)	+	D	No	see discussion for USEPA interpretation of Strain A mouse data
2,4-Dinitrotoluene	-	--	No	No
2,4-Diaminotoluene	-	--	No	No
Acrylamide	+	B2	Yes	No
Bis(chloromethyl)ether	+	A	Yes	No
1,4-Dioxane	-	B2	Yes	No

1- "+" indicates positive result in test, "-" indicates negative result in test
2- Group A – known human carcinogen
Group B2- probable human carcinogen based on animal studies
Group C- possible human carcinogen based on limited animal data
Group D- not classifiable as to human carcinogenicity
3- Chemicals with a cancer slope factor in the IRIS database are classified as "Yes".
Chemicals with no USEPA-derived slope factor on IRIS are classified as "No."